

WE CLAIM

- Sub B1
1. Recombinant CSF protein.
 2. CSF protein having a specific activity of 1×10^7 units/mg in the bone marrow assay.
 3. CSF protein as claimed in claim 2 which is recombinant CSF protein.
 4. CSF protein as claimed in claim 1 which is human granulocyte-macrophage CSF.
 5. CSF protein as claimed in claim 1 which has the amino acid sequence shown for CSF-thr in Fig. 1, or CSF-ile in Fig. 1, or CSF-G in Fig. 1.
 6. A CSF protein as claimed in claim 1 which contains the amino acid sequence as shown in Fig. 1 commencing with Ala-Pro ... or wherein the amino acid sequence commencing Ala-Pro ... is preceded by a methionine residue.
 7. A CSF protein according to claim 1 which is a CSF protein corresponding in amino acid sequence to a naturally occurring CSF, except that one or more amino acids has been added, substituted or removed without substantially affecting the biological activity of the natural CSF.
 8. A CSF protein according to claim which is a CSF protein having the amino acid sequence of a natural CSF except that it is preceded by a methionine residue.
- Sub B2

9. A method of producing a CSF protein as claimed in claim 1 which comprises isolating said CSF protein as expressed from eukaryotic or prokaryotic host cells into which has been transformed a vector, said vector having inserted therein a gene coding for said CSF protein.

10. A method as claimed in claim 9 wherein expression occurs from an E. coli, CHO or yeast.

11. A method for preparing and isolating a transformation vector containing CSF/cDNA, said method comprising:

- preparing RNA from a cell that produces CSF;
- preparing polyadenylated messenger RNA from said RNA;
- preparing single stranded cDNA from said messenger RNA;
- converting the single stranded cDNA to double stranded

cDNA;

inserting the double stranded cDNA into transformation vectors and transforming bacteria with said vector to form colonies;

picking pools of 200 to 500 colonies each and isolating plasmid DNA from each pool;

transfecting the plasmid DNA into suitable host cells for expressing CSF protein;

culturing the transfected cells and assaying the supernatant for CSF activity; and

selecting CSF positive pools and screening the colonies used to make the pool to identify a colony having CSF activity.

✓12. A method for purifying CSF protein from a mixture of proteins suspended in aqueous medium, said method comprising:
precipitating the protein with ammonium sulfate at 80% saturation to form a pellet containing the CSF protein;
resuspending the pellet in a buffered solution at a pH in the range of about 6 to about 8;
applying the buffered solution containing CSF to a chromatographic column, eluting the CSF activity with the buffered solution containing sodium chloride and collecting the fractions having CSF activity; and
pooling the active fractions, applying the pooled fractions to a C4 reverse phase column and eluting the CSF activity with a 0 to 90% acetonitrile gradient to collect the fractions containing CSF activity.

13. cDNA, or an expression vector coding for CSF according to claim 1.

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14. A pharmaceutical composition comprising a CSF according to claim 1, or CSF according to claim 1 for use in therapy.

15. A method of treating infection or granulocytopenia or activating neutrophils in animals which comprises administering CSF of claim 1 to an animal in need of much treatment, or use of CSF according to claim 1 for use in the manufacture of pharmaceutical compositions for such method.

16. A pharmaceutical composition comprising a CSF prepared according to the process of claim 12.

17. A method of treating infection or granulocytopenia or activating neutrophils in animals which comprises administering CSF of claim 12 to an animal in need of such treatment, or use of CSF according to claim 12 for use in the manufacture of pharmaceutical compositions for such method.

18. CSF protein obtainable by expressing E. coli MC1061 deposited in the ATCC under the number ATCC 39754 or the 127 amino acid CSF coding nucleotide sequence of plasmid p91023(B)-CSF deposited therein.